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Application of induced pluripotent stem cells to primary immunodeficiency diseases

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Primary immunodeficiency diseases (PIDs) are a heterogeneous group of rare immune disorders with genetic causes. Effective treatments using hematopoietic stem cells or pharmaceutical agents have been around for decades. However, for many patients, these treatment options are ineffective, partly because the rarity of these PIDs complicates the diagnosis and therapy. Induced pluripotent stem cells (iPSCs) offer a potential solution to these problems. The proliferative capacity of iPSCs allows for the preparation of a large, stable supply of hematopoietic cells with the same genome as the patient, allowing for new human cell models that can trace cellular abnormalities during the pathogenesis and lead to new drug discovery. PID models using patient iPSCs have been instrumental in identifying deviations in the development or function of several types of immune cells, revealing new molecular targets for experimental therapies. These models are only in their early stages and for the most part have recapitulated results from existing models using animals or primary cells. However, iPSC-based models are being used to study complex diseases of other organs, including those with multigenic causes, suggesting that advances in differentiation processes will expand iPSC-based models to complex PIDs as well.

Primary immunodeficiency diseases (PIDs) describe a group of clinically and genetically heterogeneous disorders that afflict lymphoid and myeloid lineages. PIDs have been recognized for a century, but the first molecular cause was not reported until 1972, when scientists realized that a child with severe combined immune deficiency (SCID) completely lacked adenosine deaminase, eventually leading to enzyme replacement therapy [1,2]. There are now a number of therapeutic options for PIDs, including cytokine-based strategies, gene therapies, and hematopoietic stem cell (HSC) transplantation [3–5]. Mutations in more than 300 genes have been associated with PIDs, but estimates assume thousands more are still to be identified [6]. Although most categorized PIDs are monogenic, advanced diagnostics are revealing that multigenic factors and somatic mosaicisms are also contributing factors. The rates of PIDs vary, with frequencies approaching as low as one in a million, and approximately 20% of PID cases were originally reported in single patients [7,8]. Reduced costs and increased speeds have made next-generation sequencing (NGS) the standard for PID diagnosis; however, a considerable number of patients with unknown genetic etiology cannot be diagnosed due to the rarity of patients or the nature of the mutation (e.g., deep intronic mutations) [9]. Furthermore, findings should be confirmed with cell assays, which can be difficult to establish using primary hematopoietic cells due to the patient's condition. The result is delayed diagnosis, which complicates treatment and worsens morbidity and mortality.

Induced pluripotent stem cells (iPSCs) provide a new model for studying these challenging PIDs. iPSCs are somatic cells reprogrammed to the pluripotent state, thus making it possible to prepare an indefinite number of cells for disease study even in circumstances when cell accessibility is difficult or only few patients are available. Importantly, these reprogrammed cells share the same genome of inaccessible primary cells. The result is new understanding of the disease pathogenesis and new molecular targets for treatment. Indeed, iPSC models have already been constructed for several PIDs, either affirming or challenging current theories about the molecular disorders contributing to the disease.

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PID modeling with iPSCs

PIDs are a wide class and primarily associated with mutations that disrupt the development or function of leukocytes, but also with inborn errors of metabolism [10]. Depending on the severity, patients can show vulnerability to a whole range of infections (e.g., *IL2RG* mutations in SCID) or in some cases to just a few viruses (e.g., *TLR3* mutations in herpes simplex encephalitis) [11]. Diagnostics include functional cell assays and genomic screenings. Complicating treatment is that any given PID can be heterogeneous and have mutations in dozens of genes without any specific set appearing causative [12]. Some patients are at extreme risk even from birth. The continuing reduction of NGS costs has made newborn screenings a standard option in suspected cases even when there is no family history [13]. Indeed, NGS has revealed that PIDs, which were once considered an exclusively Mendelian and monogenic family, are far more heterogeneous and include many multigenic and somatic factors [14]. However, despite these advances, several patients escape positive diagnosis. In these cases, when a PID is suspected but no clear genetic cause is found, autologous iPSCs should be considered. Using iPSCs from patients, scientists have gained new insights on how different cell types are affected by a PID and the developmental stage in which the disease phenotype emerges [15,16].

iPSCs describe the reprogramming of somatic cells into an embryonic stem cell (ESC)-like state. ESCs are pluripotent and can replicate indefinitely. They and, by extension, iPSCs can model the development of all three germ layers, including all stages of hematopoiesis [17]. Exploiting these features, researchers have induced iPSCs to differentiate into an assortment of hematopoietic cells [18]. The first iPSCs were generated by exogenously expressing a master set of transcription factors (*Oct3/4, Sox2, Klf4*, and *c-Myc*) [19,20]. Since the original iPSC studies, this master set has been used to prepare iPSCs from multiple types of somatic cells from multiple types of species, confirming universality of the reprogramming mechanism.

Just as there are species differences in embryonic and hematopoietic development, there are species differences in ESCs and iPSCs (collectively, pluripotent stem cells, or PSCs), namely, the quality of the pluripotency. Human PSCs have primed pluripotency, whereas mouse PSCs have naïve pluripotency [21]. The different states describe the stability of the pluripotency and have implications on the creation of chimeric animals. Researchers have managed to reset primed human iPSCs to the naïve state, but the chimeric potential of these cells has never been confirmed and, unlike mouse PSCs, no naïve human PSCs have been generated without first acquiring the primed state [22,23]. The pluripotency state is considered a key determinant for modeling early development. Therefore, although hematopoietic cells have been successfully generated from human primed PSCs, the quality of the cells may be improved if naïve PSCs are the starting source.

Aside from the development potential of iPSCs, they provide a unique model that can recapitulate disease phenotypes in vitro. Within 2 years of the first human iPSC report, scientists confirmed that patient cells can be reprogrammed to iPSCs, which were used to study the disease pathogenesis [24,25]. Moreover, patient iPSCs are the basis for several clinical cell therapies and drug studies (Table 1). Not included in these lists is an iPSC model that identified a candidate neutrophil elastase inhibitor that could recover mature neutrophils

Table 1. iPSCs in the clinical setting

Disease	Type of Therapy	Status
iPSC-based cell therapies		
Age-related macular degeneration [73]	Autologous and allogeneic transplantation of iPSC-based product	<10 patients have received transplantation of retinal epithelial cells
Aplastic anemia with platelet refractoriness [64]	Autologous transfusion of iPSC-derived platelets	Single patient has been recruited; awaiting regulatory approval
Heart failure [74]	Allogeneic transplantation of iPSC-based product	Patient recruitment
Parkinson's disease [75]	Allogeneic transplantation of iPSC-based product	First operation was done in November 2018
Spinal cord therapy [76]	Allogeneic transplantation of iPSC-based product	Approval received from university board of ethics; approval from the Japan national regulatory body is expected
Drug trials based on iPSC patient models		
Amyotrophic lateral sclerosis [77]	Drug (ergobine)	Phase II
Fibrodysplasia ossifcans progressiva [78]	Drug (rapamycin)	<20 patients have received the drug in double-blind study
Progressive supernuclear palsy [79]	Drug (BMS-986168)	Phase II
Spinal muscular atrophy [80]	Drug (RG7800)	Terminated after Phase I

from patients with severe congenital neutropenia (SCN) and who are unresponsive to alternative treatments [26]. Important from an industry perspective, some of these drug candidates have come not from drug discovery, but rather from drug repositioning, which is estimated to lower the cost and time to approval by one-third [27].

Accordingly, several patient iPSC models have been made to investigate blood diseases, including those causing bone marrow failure, anemia, and leukemia [28]. These studies have not yet led to clinical application, but they have revealed insights about which developmental stage the disease first manifests. For example, reprogramming cells from Fanconi anemia patients identified the disturbed differentiation capacity associated with the disease to occur as early as the hemoangiogenic progenitor stage, biasing cells to differentiate away from hematopoietic lineages and toward endothelial lineages [29]. Another study using iPSCs from Diamond–Blackfan anemia patients found that erythropoiesis could be enhanced by a small chemical that induced autophagy via ATG5, a molecule required for autophagosome formation [30]. Another SCN study found that the efficiency of neutrophil differentiation from patient iPSCs with a mutation in *HAX1* was 25% that of healthy donor cells [31]. Instead, the differentiation tended to arrest at the myeloid progenitor stage and showed a higher propensity for apoptosis, consistent with the abnormal granulopoiesis seen in the disease. The transduction of *HAX1* by lentivirus recovered the low neutrophil count.

Fortunately, we have had exceptional access to consenting patients suffering from rare PIDs, allowing us to reprogram their cells and build models to examine monocyte and macrophage development. The findings from these investigations have confirmed other models or revealed previously unknown molecular causes (see below for examples).

Mutations to *NOD2* are the cause of Blau syndrome, a congenital monogenic granulomatosis. NOD2 is a receptor that upon binding to muramyl dipeptide (MDP) activates the nuclear factor-kappa beta (NF-κB) pathway, upregulating cytokines and chemokines [32,33]. We reprogrammed cells from two NOD2 patients with the R334W mutation. In one group of iPSCs, the mutation was corrected by gene editing. In addition, cells from a healthy donor were reprogrammed with the mutation knocked in. Assays revealed distinctive responses by iPSC-derived macrophages to interferon-gamma (IFN-γ) stimulation based on the mutation [34]. In all cases, IFN-γ stimulation upregulated NOD2 expression, indicating a priming role. However, only in cells derived from mutant iPSCs did IFN-γ alone activate NF-κB (Figure 1A). In the corrected case, MDP binding was required after the IFN-γ priming signal, suggesting that targeting IFN-γ could have therapeutic benefit. These results were confirmed in primary patient macrophages.

Neonatal-onset multisystem inflammatory disease (NOMID), or chronic infantile neurologic, cutaneous and arthritis (CINCA), is the most clinically severe form of cryopyrin-associated periodic syndrome. The autoinflammation primarily affects the nerves, skin, and joints, and patients will develop sensory problems due to meningitis, rashes, and joint deformities. The majority of cases are associated with a mutation in the *NLRP3* gene, which causes excessive secretion of interleukin-1 beta (IL-1β) by monocytes [35,36]. Dysfunction in the NLRP3 inflammasome is associated with several metabolic diseases such as gout and obesity, and many clinical therapies target the excessive IL-1β or the NLRP3 complex [37].

However, a large minority of NOMID patients do not express a pathological mutation in *NLRP3*, instead showing somatic mosaicism. One such case was shown in a single Japanese patient with a mutation in *NLRC4* [38]. Interest in this gene has risen in recent years due to its discovered role in several autoinflammatory syndromes [39–41]. Fibroblasts from the NOMID patient were reprogrammed into iPSCs and then differentiated into monocytes. Consistent with NOMID symptoms, the iPSC-derived monocytes secreted IL-1β without a secondary signal. Interestingly, activation absent the secondary signal was true of only some iPSC clones despite all being made from the same patient (Figure 1B). Whole-exome sequence analysis of the two clone types revealed a somatic mosaicism of the *NLRC4* mutation T177A as causative. Correcting the mutation by gene editing resulted in monocytes that behaved normally. Many NOMID patients are diagnosed without identifying causal mutations and somatic mutations can occur at frequencies below the detection limit of NGS systems, reiterating the benefits of iPSCs for rare PIDs [42].

Nakajo–Nishimura syndrome (NNS) is an autosomal disorder caused by mutations in *PSMB8*, which encodes for β5i protein, one of three subunits specialized for the immunoproteosome [43,44]. Point mutations in this gene are associated with phenotypes beyond autoimmune symptoms, including muscle atrophy and lipodystrophy [45,46]. NNS patients are susceptible to rashes and skin eruptions and are usually treated with corticosteroids, but this therapy does not ameliorate the lipodystrophy [47]. In NNS, the upregulation of proinflammatory factors is attributed to the p38 mitogen-activated protein kinase (MAPK) pathway. As expected, myeloid cells differentiated from patient iPSCs showed reduced immunoproteasome activity, higher production of reactive oxygen species, and increased production of IL-6, MCP-1, and IP-10, cytokines and chemokines that are associated with the

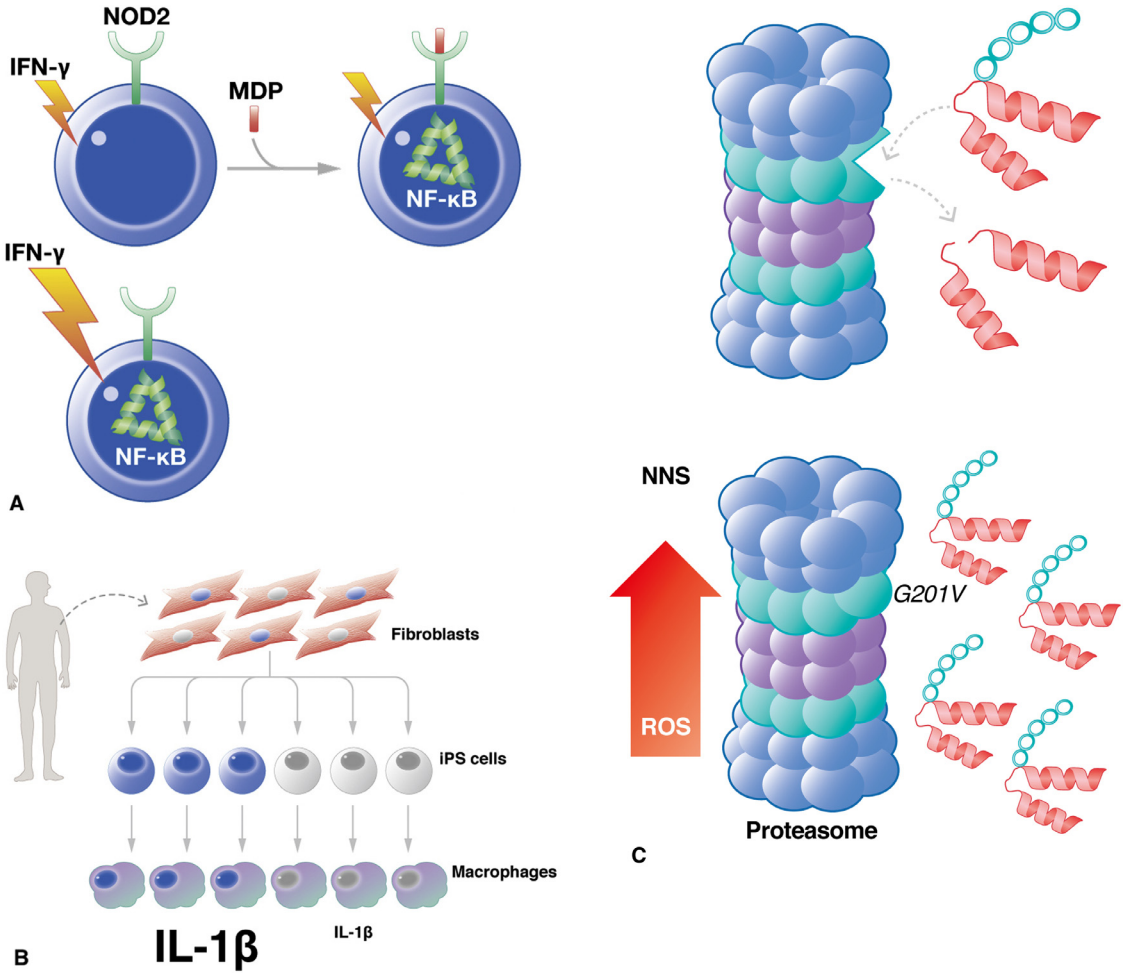

Figure 1. Patient iPSC models for PIDs. **(A)** Mutations in *NOD2* are a genetic cause of Blau syndrome. In normal conditions, IFN-γ acts as the priming signal in macrophages. The activating signal comes from MDP binding to the NOD2 receptor. Macrophages differentiated from patient iPSCs showed that IFN-γ acts as both the priming and activating signal, thus activating NF-κB signaling even without MPD binding. **(C)** A point mutation in PSMB8 (G201V) is the genetic cause of NNS. This mutation is believed to result in a structural change in the immunoproteosome. Myeloid cells differentiated from NNS-patient iPSCs showed reduced immunoproteosome activity. As a result, the myeloid cells produced excessive reactive oxygen species (ROS), causing the cells to secrete the inflammatory factors IL-6, MCP-1, and IP-10. The ribbon structures show processed proteins by normal immunoproteosome (top) and unprocessed proteins by mutant immunoproteosome (bottom), the latter causing ROS. **(B)** For most patients, the genetic cause of NOMID is attributed to a mutation in *NLRP3*. However, for some patients, no mutation in this gene exists. A NOMID iPSC model revealed that a subset of monocytes had a point mutation in *NLRC4* (cells with blue nuclei) and secreted IL-1β without a secondary signal. Other monocytes without the *NLRC4* mutation (cells with grey nuclei) did not express this phenotype.

disease [48]. Furthermore, myeloid cells from patient and isogenic control iPSCs differed in that the mutant types showed evidence of being in a primed state even absent a stimulus (Figure 1C). Application of a p38 MAPK inhibitor to the cells suppressed the secretion of IL-6, MCP-1, and IP-10 in a dose-dependent manner, demonstrating how patient iPSCs in combination with gene editing for the generation of isogenic controls could contribute to drug discovery for PIDs.

Some PIDs associated with mutations in proteasome dysfunctions, like those seen with *PSMB8* mutations, are multigenic [49]. Such diseases have proven a degree more difficult to study with iPSCs. However, success has been reported with complex disorders such as autism or idiopathic conditions such as amyotrophic lateral sclerosis, suggesting that a combination of iPSCs with other biotechnologies such as gene editing could lead to major breakthroughs for this category of PIDs [50,51]. Additionally, some PIDs are attributed to epigenetic alterations without any known genetic origin [52]. Cell reprogramming involves epigenetic resetting without changes to the genome and studies have shown how cell reprogramming in vivo can be used to study epigenetic causes in solid cancers [53]. Human iPSCs only recently reached their second decade and current disease models for PIDs could be viewed as a first

generation that complements existing cellular or animal models. Studies that build on models for other organs or with other technologies should advance the study of complex PIDs.

Generating monocytes and macrophages for disease study

Ideally, the differentiation of iPSCs to a specific cell type will follow the natural developmental program of the embryo. However, limited understanding of tissue development means that sometimes the induced cells do not recapitulate the function or maturity of primary cells, compromising the quality of the model. Most monocytes and macrophages develop from definitive hematopoiesis in the adult, although some tissue-resident macrophages such as microglia emerge from primitive hematopoiesis [54]. Macrophage subtypes can be acquired by coculturing the iPSCs with other cells in the tissue microenvironment [55]. Standard differentiation protocols for macrophages from iPSCs use a cocktail of cytokines, including IL-3 and macrophage-colony stimulating factor (M-CSF), to generate macrophages from hematopoietic progenitor cells (HPCs) [56,57]. IL-3 expands the HPC population with myeloid lineage bias, and M-CSF promotes terminal macrophage differentiation [58,59]. Although the generated cells are macrophages, they can show intermediate phenotypes of different subtypes, confounding their function and thus the accuracy of the corresponding cell model, reiterating that PID models still have room for improvement [60].

Few studies have investigated the engraftment potential of PSC-differentiated monocyte and macrophage progenitors in animal models and none to our knowledge have transplanted cells derived from PID patient iPSCs. The expression of specific sets of transcription factors that enhance the engraftment potential of progenitor cells that result in myeloid lineage has been reported. HOXA9, ERG, and RORA were found to induce the self-renewal and multipotency capacity of progenitors derived from human PSCs by reactivating HSC genes [61]. The additional expression of SOX4 and MYB biased the progenitors toward erythroid and myeloid lineage upon engraftment in a mouse model. Building on that study, the same group showed that human PSCs could generate HPCs with good engraftment potential and reconstitute the myeloid and lymphoid in mice by overexpressing seven transcription factors (*ERG, HOXA5, HOX9, HOXA10, LCOR, RUNX1*, and *SPI1*), all of which have recognized roles in HSC development, maintenance, or lineage commitment [62]. Current PID iPSC models do not consider the expression of these factors in the differentiation protocol. Therefore, whereas the disease phenotype is expressed in cell assays, there is no confirmation that these cells recapitulate the disease in animal models.

Furthermore, protocols that induce the myeloid differentiation of PSCs give comparably more consideration to the biochemistry (cytokines) than the biophysics (fluid dynamics) of the microenvironment. More than other tissues, blood cells operate in a very dynamic environment and consideration of the physical parameters in the culture system is expected to enhance the quality of the differentiated cells. In one example, megakaryocytes were induced from iPSCs by overexpressing *c-MYC, BMI1*, and *BCL-XL* [63]. These megakaryocytes shed functional platelets, but their ploidy and low platelet output suggested that they were immature. Revision to a bioreactor that introduced both laminar flow and localized turbulent flow around the megakaryocytes resulted in a platelet count sufficient for transfusion therapy, and this project is now heading toward a clinical trial [64]. Currently, organoid technologies have arguably the best potential for recreating the chemical and physical microenvironment because they attempt to recapture the patterning and intermediate stages during natural development that lead to the final cell state [65].

Another factor affecting the quality of myeloid induction from PSCs may be the markers that we use to identify them. Typically, cell populations are defined by surface markers, but intracellular markers may prove more reliable, especially with regard to maturity or subtype. MicroRNAs (miRNAs) are noncoding RNAs that suppress translation by binding to complementary RNA and can be used as diagnostic tools for cancer and other diseases [66,67]. miRNAs have also been shown to regulate reprogramming and differentiation mechanisms, including those for myeloid and lymphoid lineages, and an analysis of miRNA expressions has indicated dynamic expressions of miRNAs during the differentiation of iPSCs to different hematopoietic cell stages [68–70]. Additionally, the overexpression of SOX2 and miR125b was found to promote the direct differentiation of human fibroblasts into macrophage progenitors, which went on to produce mature macrophages when transplanted into mice [71]. These studies suggest that miRNAs could be useful markers for progenitors and that analyzing miRNA expression could contribute to the induction of higher quality hematopoietic cells. In response, colleagues of ours have developed the miRNA switch, an RNA-based synthetic structure that turns gene expressions on or off in living cells depending on the presence of specific miRNA or RNA-binding proteins (Figure 2). By constituting miRNA switches with complementary RNA that bind to intracellular markers for different cell types, they have successfully activated apoptotic genes to eliminate unwanted cells from a heterogeneous population, thus purifying the targeted cell subpopulations [72].

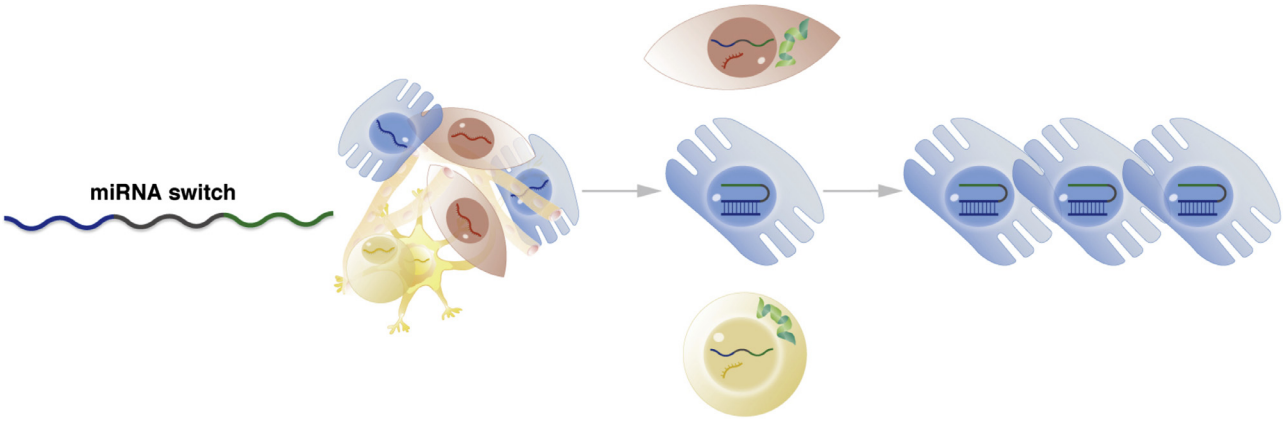

Figure 2. miRNA switches. miRNA switches use intracellular markers to turn genes on or off. In the schematic, the miRNA switch is a single-strand RNA that includes the sequence of an apoptotic gene (green) and a sequence (blue) complementary to miRNA that is highly expressed in blue cells but not in other cells. Binding of the miRNA to the complementary sequence causes a structural change in the mRNA that prevents the apoptotic protein from being expressed (green ribbon in yellow and red cells). Therefore, when this miRNA switch is transfected into the shown heterogeneous cell population, only the blue cells survive because their miRNA binds to the switch to inhibit protein synthesis (the loop in the RNA structure indicates a conformational change blocking translation). This approach allows for the selection of living cells with specific intracellular markers.

Conclusions

Advances in gene editing, NGS, and other technologies have made PIDs manageable, if not curable, for many patients. Scientists can now obtain unprecedented detail about the molecular and genetic causes, providing new targets for gene therapy and drug testing. However, despite similar symptoms, molecular causes frequently deviate in PID patients. In these cases, especially when patients are rare, assays of patient cells can be most informative, but sometimes primary cells are difficult to procure or simply are not informative. Accordingly, cell reprogramming, namely iPSCs, brings a revolutionary framework to the study of PIDs. Current models are still relatively simple and for the most part have confirmed other cell and animal models. However, as we clarify hematopoiesis and adjust differentiation protocols accordingly, patient iPSC models, which retain the genome of the primary cells, are expected to open the door to a new generation of cell and drug therapies.

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References

1. Giblett ER, Anderson JE, Cohen F, et al. Adenosine-deaminase deficiency in two patients with severely impaired cellular immunity. Lancet. 1972;2:1067–1069.
2. Ochs HD, Petroni D. From clinical observations and molecular dissection to novel therapeutic strategies for primary immunodeficiency disorders. Am J Med Genet A. 2018;176:784–803.
3. Gatti RA, Meuwisse Hj, Allen HD, et al. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet. 1968;2:1366–1369.
4. Marciano BE, Holland SM. Primary immunodeficiency diseases: current and emerging therapeutics. Front Immunol. 2017;8:937.
5. Thrasher AJ, Williams DA. Evolving gene therapy in primary immunodeficiency. Mol Ther. 2017;25:1132–1141.
6. Itan Y, Casanova JL. Novel primary immunodeficiency candidate genes predicted by the human gene connectome. Front Immunol. 2015;6:142.
7. Casanova JL, Conley ME, Seligman SJ, et al. Guidelines for genetic studies in single patients: lessons from primary immunodeficiencies. J Exp Med. 2014;211:2137–2149.
8. Fang M, Abolhassani H, Lim CK, et al. Next generation sequencing data analysis in primary immunodeficiency disorders: future directions. J Clin Immunol. 2016;36(Suppl 1):68–75.
9. Boisson B, Honda Y, Ajiro M, et al. Rescue of recurrent deep intronic mutation underlying cell type-dependent quantitative NEMO deficiency. J Clin Invest. 2019;129(2):15.
10. Parvaneh N, Quartier P, Rostami P, et al. Inborn errors of metabolism underlying primary immunodeficiencies. J Clin Immunol. 2014;34:753–771.
11. Fodil N, Langlais D, Gros P. Primary immunodeficiencies and inflammatory disease: a growing genetic intersection. Trends Immunol. 2016;37:126–140.
12. Walkovich K, Connelly JA. Primary immunodeficiency in the neonate: early diagnosis and management. Semin Fetal Neonatal Med. 2016;21:35–43.
13. Pavey AR, Bodian DL, Vilboux T, et al. Utilization of genomic sequencing for population screening of immunodeficiencies in the newborn. Genet Med. 2017;19:1367–1375.

14. Seleman M, Hoyos-Bachiloglu R, Geha RS, et al. Uses of next-generation sequencing technologies for the diagnosis of primary immunodeficiencies. Front Immunol. 2017;8:847.
15. Menon T, Firth AL, Scripture-Adams DD, et al. Lymphoid regeneration from gene-corrected SCID-X1 subject-derived iPSCs. Cell Stem Cell. 2015;16:367–372.
16. Zimmer B, Ewaleifoh O, Harschnitz O, et al. Human iPSC-derived trigeminal neurons lack constitutive TLR3-dependent immunity that protects cortical neurons from HSV-1 infection. Proc Natl Acad Sci U S A. 2018;115:E8775–E8782.
17. Wahlster L, Daley GQ. Progress towards generation of human haematopoietic stem cells. Nat Cell Biol. 2016;18:1111–1117.
18. Karagiannis P, Takahashi K, Saito M, et al. Induced pluripotent stem cells and their use in human models of disease and development. Physiol Rev. 2019;99:79–114.
19. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131:861–872.
20. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006;126:663–676.
21. Nichols J, Smith A. Naive and primed pluripotent states. Cell Stem Cell. 2009;4:487–492.
22. Takashima Y, Guo G, Loos R, et al. Resetting transcription factor control circuitry toward ground-state pluripotency in human. Cell. 2014;158:1254–1269.
23. Theunissen TW, Powell BE, Wang H, et al. Systematic identification of culture conditions for induction and maintenance of naive human pluripotency. Cell Stem Cell. 2014;15:471–487.
24. Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. Science. 2008;321:1218–1221.
25. Lee G, Papapetrou EP, Kim H, et al. Modelling pathogenesis and treatment of familial dysautonomia using patient-specific iPSCs. Nature. 2009;461:402–406.
26. Makaryan V, Kelley ML, Fletcher B, et al. Elastase inhibitors as potential therapies for ELANE-associated neutropenia. J Leukoc Biol. 2017;102:1143–1151.
27. Karagiannis P, Onodera A, Yamanaka S. New models for therapeutic innovation from japan. EBioMedicine. 2017;18:3–4.
28. Dolatshad H, Tatwavedi D, Ahmed D, et al. Application of induced pluripotent stem cell technology for the investigation of hematological disorders. Adv Biol Regul. 2019;71:19–33.
29. Suzuki NM, Niwa A, Yabe M, et al. Pluripotent cell models of fanconi anemia identify the early pathological defect in human hemoangiogenic progenitors. Stem Cells Transl Med. 2015;4:333–338.
30. Doulatov S, Vo LT, Macari ER, et al. Drug discovery for Diamond–Blackfan anemia using reprogrammed hematopoietic progenitors. Sci Transl Med. 2017;9:eaah5645.
31. Morishima T, Watanabe K, Niwa A, et al. Genetic correction of HAX1 in induced pluripotent stem cells from a patient with severe congenital neutropenia improves defective granulopoiesis. Haematologica. 2014;99:19–27.
32. Girardin SE, Boneca IG, Viala J, et al. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. J Biol Chem. 2003;278:8869–8872.
33. Inohara N, Ogura Y, Fontalba A, et al. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. J Biol Chem. 2003;278:5509–5512.
34. Takada S, Kambe N, Kawasaki Y, et al. Pluripotent stem cell models of Blau syndrome reveal an IFN-gamma-dependent inflammatory response in macrophages. J Allergy Clin Immunol. 2018;141:339–349.e311.
35. Tanaka N, Izawa K, Saito MK, et al. High incidence of NLRP3 somatic mosaicism in patients with chronic infantile neurologic, cutaneous, articular syndrome: results of an International Multicenter Collaborative Study. Arthritis Rheum. 2011;63:3625–3632.
36. Goldbach-Mansky R. Current status of understanding the pathogenesis and management of patients with NOMID/CINCA. Curr Rheumatol Rep. 2011;13:123–131.
37. Shao BZ, Xu ZQ, Han BZ, et al. NLRP3 inflammasome and its inhibitors: a review. Front Pharmacol. 2015;6:262.
38. Kawasaki Y, Oda H, Ito J, et al. Identification of a high-frequency somatic NLRC4 mutation as a cause of autoinflammation by pluripotent cell-based phenotype dissection. Arthritis Rheumatol. 2017;69:447–459.
39. Canna SW, de Jesus AA, Gounil S, et al. An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome. Nat Genet. 2014;46:1140–1146.
40. Kitamura A, Sasaki Y, Abe T, et al. An inherited mutation in NLRC4 causes autoinflammation in human and mice. J Exp Med. 2014;211:2385–2396.
41. Romberg N, Al Moussawi K, Nelson-Williams C, et al. Mutation of NLRC4 causes a syndrome of enterocolitis and autoinflammation. Nat Genet. 2014;46:1135–1139.
42. Hoffman HM, Broderick L. Editorial: it just takes one: somatic mosaicism in autoinflammatory disease. Arthritis Rheumatol. 2017;69:253–256.
43. Arima K, Kinoshita A, Mishima H, et al. Proteasome assembly defect due to a proteasome subunit beta type 8 (PSMB8) mutation causes the autoinflammatory disorder, Nakajo–Nishimura syndrome. Proc Natl Acad Sci U S A. 2011;108:14914–14919.
44. Murata S, Yashiroda H, Tanaka K. Molecular mechanisms of proteasome assembly. Nat Rev Mol Cell Biol. 2009;10:104–115.
45. Agarwal AK, Xing C, DeMartino GN, et al. PSMB8 encoding the beta5i proteasome subunit is mutated in joint contractures, muscle atrophy, microcytic anemia, and panniculitis–induced lipodystrophy syndrome. Am J Hum Genet. 2010;87:866–872.
46. Kitamura A, Maekawa Y, Uehara H, et al. A mutation in the immunoproteasome subunit PSMB8 causes autoinflammation and lipodystrophy in humans. J Clin Invest. 2011;121:4150–4160.
47. Kunimoto K, Kimura A, Uede K, et al. A new infant case of Nakajo–Nishimura syndrome with a genetic mutation in the immunoproteasome subunit: an overlapping entity with JMP and CANDLE syndrome related to PSMB8 mutations. Dermatology. 2013;227:26–30.
48. Honda-Ozaki F, Terashima M, Niwa A, et al. Pluripotent stem cell model of Nakajo–Nishimura syndrome untangles proinflammatory pathways mediated by oxidative stress. Stem Cell Reports. 2018;10:1835–1850.
49. Brehm A, Liu Y, Sheikh A, et al. Additive loss-of-function proteasome subunit mutations in CANDLE/PRAAS patients promote type I IFN production. J Clin Invest. 2015;125:4196–4211.
50. Marchetto MC, Belinson H, Tian Y, et al. Altered proliferation and networks in neural cells derived from idiopathic autistic individuals. Mol Psychiatry. 2017;22:820–835.
51. Imamura K, Izumi Y, Watanabe A, et al. The Src/c-Abl pathway is a potential therapeutic target in amyotrophic lateral sclerosis. Sci Transl Med. 2017;9:eaaf3962.
52. Campos-Sanchez E, Martinez-Cano J, Del Pino Molina L, et al. Epigenetic deregulation in human primary immunodeficiencies. Trends Immunol. 2019;40:49–65.
53. Sogabe Y, Seno H, Yamamoto T, et al. Unveiling epigenetic regulation in cancer, aging, and rejuvenation with in vivo reprogramming technology. Cancer Sci. 2018;109:2641–2650.
54. McGrath KE, Frame JM, Palis J. Early hematopoiesis and macrophage development. Semin Immunol. 2015;27:379–387.
55. Takata K, Kozaki T, Lee CZW, et al. Induced-pluripotent-stem-cell-derived primitive macrophages provide a platform for

modeling tissue-resident macrophage differentiation and function. Immunity. 2017;47:183–198.e6.
56. Yanagimachi MD, Niwa A, Tanaka T, et al. Robust and highly-efficient differentiation of functional monocytic cells from human pluripotent stem cells under serum- and feeder cell-free conditions. PLoS One. 2013;8:e59243.
57. Higaki K, Hirao M, Kawana-Tachikawa A, et al. Generation of HIV-resistant macrophages from IPSCs by using transcriptional gene silencing and promoter-targeted RNA. Mol Ther Nucleic Acids. 2018;12:793–804.
58. Clark SC, Kamen R. The human hematopoietic colony-stimulating factors. Science. 1987;236:1229–1237.
59. Donahue RE, Seehra J, Metzger M, et al. Human IL-3 and GM-CSF act synergistically in stimulating hematopoiesis in primates. Science. 1988;241:1820–1823.
60. Lachmann N, Ackermann M, Frenzel E, et al. Large-scale hematopoietic differentiation of human induced pluripotent stem cells provides granulocytes or macrophages for cell replacement therapies. Stem Cell Reports. 2015;4:282–296.
61. Doulatov S, Vo LT, Chou SS, et al. Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via respecification of lineage–restricted precursors. Cell Stem Cell. 2013;13:459–470.
62. Sugimura R, Jha DK, Han A, et al. Haematopoietic stem and progenitor cells from human pluripotent stem cells. Nature. 2017;545:432–438.
63. Nakamura S, Takayama N, Hirata S, et al. Expandable megakaryocyte cell lines enable clinically applicable generation of platelets from human induced pluripotent stem cells. Cell Stem Cell. 2014;14:535–548.
64. Ito Y, Nakamura S, Sugimoto N, et al. Turbulence activates platelet biogenesis to enable clinical scale ex vivo production. Cell. 2018;174:636–648.e18.
65. Takebe T, Wells JM, Helmrath MA, et al. Organoid center strategies for accelerating clinical translation. Cell Stem Cell. 2018;22:806–809.
66. Lin RJ, Lin YC, Chen J, et al. microRNA signature and expression of Dicer and Drosha can predict prognosis and delineate risk groups in neuroblastoma. Cancer Res. 2010;70:7841–7850.
67. Romaine SPR, Tomaszewski M, Condorelli G, et al. MicroRNAs in cardiovascular disease: an introduction for clinicians. Heart. 2015;101:921–928.
68. Ferreira AF, Calin GA, Picanco-Castro V, et al. Hematopoietic stem cells from induced pluripotent stem cells: considering the role of microRNA as a cell differentiation regulator. J Cell Sci. 2018;131:jcs203018.
69. Gentner B, Visigalli I, Hiramatsu H, et al. Identification of hematopoietic stem cell–specific miRNAs enables gene therapy of globoid cell leukodystrophy. Sci Transl Med. 2010;2:58ra84.
70. Sweeney CL, Teng R, Wang H, et al. Molecular analysis of neutrophil differentiation from human induced pluripotent stem cells delineates the kinetics of key regulators of hematopoiesis. Stem Cells. 2016;34:1513–1526.
71. Pulecio J, Nivet E, Sancho-Martinez I, et al. Conversion of human fibroblasts into monocyte–like progenitor cells. Stem Cells. 2014;32:2923–2938.
72. Miki K, Endo K, Takahashi S, et al. Efficient detection and purification of cell populations using synthetic microRNA switches. Cell Stem Cell. 2015;16:699–711.
73. Mandai M, Watanabe A, Kurimoto Y, et al. Autologous induced stem-cell-derived retinal cells for macular degeneration. N Engl J Med. 2017;376:1038–1046.
74. Miyagawa S, Sawa Y. Building a new strategy for treating heart failure using induced pluripotent stem cells. J Cardiol. 2018;72:445–448.
75. Kikuchi T, Morizane A, Doi D, et al. Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. Nature. 2017;548:592–596.
76. Nagoshi N, Okano H. iPSC-derived neural precursor cells: potential for cell transplantation therapy in spinal cord injury. Cell Mol Life Sci. 2018;75:989–1000.
77. McNeish J, Gardner JP, Wainger BJ, et al. From dish to bedside: lessons learned while translating findings from a stem cell model of disease to a clinical trial. Cell Stem Cell. 2015;17:8–10.
78. Hino K, Horigome K, Nishio M, et al. Activin-A enhances mTOR signaling to promote aberrant chondrogenesis in fibrodysplasia ossificans progressiva. J Clin Invest. 2017;127:3339–3352.
79. Bright J, Hussain S, Dang V, et al. Human secreted tau increases amyloid–beta production. Neurobiol Aging. 2015;36:693–709.
80. Naryshkin NA, Weetall M, Dakka A, et al. Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. Science. 2014;345:688–693.